THE EFFECT OF SUBLETHAL CONCENTRATION OF ABATE ON *Aedes aegypti* (Linnaeus) AND *Culex quinquefasciatus* (Says)

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by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATION

The following abbreviations have been used commonly throughout this thesis:

DDT	Dichoro-diphenyl-tricholoethane
DF	Dengue fever
DHF	Dengue hemorrhagic fever
IGR	Insect growth regulator
LC	Lethal concentration
SE	Standard error
mg/l	milligram per liter
P	Significant
n	Number of samples

KAJIAN KESAN SUBMAUT ABATE KE ATAS *AEDES AEGYPTI* (LINNAEUS) DAN *CULEX QUINQUEFASCIATUS* (SAYS)

ABSTRAK

Kajian keberkesanan dan kesan submaut Abate ke atas beberapa parameter biologi *Aedes aegypti* and *Culex quinquefasciatus* telah dijalankan di makmal menggunakan teknik bioasei WHO. Larva instar tahap lewat ketiga telah didedahkan kepada larvIsid selama dua puluh empat jam. Keputusan probit untuk nilai LC_{50} dan LC_{95} untuk *Aedes aegypti* ialah 0.0006 mg/l and 0.001mg/l, masing-masingnya dengan slop regresi 7.30 ± 0.60, manakala nilai LC_{50} dan LC_{95} bagi *Culex quinquefasciatus* ialah 0.00088 mg/l and 0.00249 mg/l, masing-masingnya dengan slop regresi 3.64 ± 0.22.

Larva *Aedes aegypti* yang dirawat dengan Abate pada kepekatan submaut menunjukkan tempoh peringkat larva induk (P) yang panjang; begitu juga dengan induk (P) dan F1 *Culex quinquefasciatus.* Generasi F2 *Aedes aegypti* dan F1 *Culex quinquefasciatus* yang dirawat menunjukkan peningkatan yang signifikan (P<0.05) dalam jumlah telur yang dihasilkan. Min bilangan telur generasi F2 dan F3 *Aedes aegypti* menunjukkan perbezaan yang signifikan. Generasi F1 *Culex quinquefasciatus* juga menunjukkan peningkatan yang signifikan (P<0.05) dalam bilangan telur yang dihasilkan. Telur generasi F1 dan F2 *Aedes aegypti* dan generasi F1 dan F3 *Culex quinquefascaitus* yang dirawat menunjukkan penurunan yang signifikan (P<0.05) dalam peratusan penetasan. Min kemunculan dewasa generasi F3 betina dan jantan *Aedes aegytpi* dan generasi F1 dan F2 betina *Culex quinquefasciatus* yang dirawat berbeza secara signifikan (P<0.05). Peratusan mortaliti generasi F1, F2 dan F3 larva *Culex quinquefasciatus* mengalami penurunan yang signifikan (P<0.05). Pupa

generasi F1 *Culex quinquefasciatus* tidak mengalami sebarang mortaliti, manakala pupa generasi F2 menunjukkan peningkatan mortaliti yang signifikan ($P<0.05$). Pengambilan sarapan darah generasi F1 dan F2 *Culex quinquefasciatus* mengalami penurunan masing-masing sebanyak 7.3% dan 4%. Betina dan jantan induk (P) dan betina generasi F2 yang dirawat dan menghisap sarapan sukrosa menunjukkan penurunan yang signifikan ($P<0.05$) dalam tempoh hidup peringkat dewasa. Generasi F1 betina *Aedes aegypti* yang dirawat, generasi F2 dan F3 betina *Culex quinquefasciatus* yang dirawat dan mengambil sarapan darah menunjukkan penurunan dalam tempoh hidup peringkat dewasa. Nisbah nyamuk jantan *Aedes aegypti* induk (P), F1, F2 dan F3 yang dirawat menunjukkan penurunan dalam nisbah jantina, manakala nisbah betina generasi F3 meningkat. Nisbah betina *Culex quinquefasciatus* induk (P) didapati meningkat, manakala nisbah jantan generasi F1 menurun.

Abate tidak memberi kesan ke atas pengambilan sarapan darah, tempoh pembentukan pupa, peratusan mortaliti larva dan pupa, tempoh hidup dewasa jantan dan betina *Aedes aegypti* yang mengambil sarapan sukrosa; dan juga min masa kemunculan jantan *Culex quinquefasciatus*. Mortaliti gerenerasi F1, F2 dan F3 bagi larva *Aedes aegypti* dan *Culex quinquefasciatus* menunjukkan penurunan peratusan selepas pendedahan kepada kepekatan submaut pada kali yang kedua.

THE EFFECT OF SUBLETHAL CONCENTRATION OF ABATE ON *AEDES AEGYPTI* (LINNAEUS) AND *CULEX QUINQUEFASCIATUS* (SAYS)

ABSTRACT

A laboratory study on the effectiveness and the sublethal effects of Abate on several biological parameters of *Aedes aegypti* and *Culex quinquefasciatus* was conducted using standard WHO bioassay technique. Late third instar larvae were exposed to larvacide for twenty four hours. The LC_{50} and LC_{95} obtained using Probit analysis for *Aedes aegypti* were 0.0006 mg/l and 0.001mg/l, respectively, with a regression slope of 7.30 ± 0.60, whereas for *Culex quinquefasciatus* were 0.00088 mg/l and 0.00249 mg/l, respectively with a regression slope of 3.64 ± 0.22.

Treated parent population (P) of *Aedes aegypti*, treated parent population (P) and F1 of *Culex quinquefasciatus* larvae at sublethal concentration of Abate showed prolonged larval duration. Treated F2 *Aedes aegypti* and F1 *Culex quinquefasciatus* larvae showed significant increase in the total eggs deposited. Significant differences were observed in the mean number of eggs deposited by treated F2, F3 *Aedes aegypti* and treated F1 *Culex quinquefasciatus* ($P<0.05$). Significant reductions of egg hatching percentage were found in treated F1 and F2 *Aedes aegypti* and treated F1 and F3 of *Culex quinquefascaitus* ($P<0.05$). Significant differences were observed in the mean adult emergence time of treated F3 female and male of *Aedes aegypti* and treated F1 and F2 female of *Culex quinquefasciatus* ($P<0.05$). Percentage mortality of F1, F2 and F3 *Culex quinquefasciatus* larvae were reduced significantly ($P<0.05$). No mortality was observed in F1 *Culex quinquefasciatus* pupae; F2 pupae showed significant increased in mortality ($P<0.05$). Blood

engorgement of F1 and F2 of *Culex quinquefasciatus* were reduced by 7.3% and 4% respectively. Longevity of sucrose-fed population (P) of treated *Culex quinquefasciatus* females and males and F2 females were reduced significantly ($P<0.05$). Longevity of blood-fed treated F1 *Aedes aegypti* females, treated F2 and F3 *Culex quinquefasciatus* females were reduced significantly ($P<0.05$). The proportion of *Aedes aegypti* males of the treated population (P), F1, F2 and F3 were reduced, while the proportion of F3 females increased. The female proportion of treated population (P) of *Culex quinquefasciatus* increased; the F1 male proportion was reduced. Abate did not affect blood engorgement, mean pupation time, percentage mortality of larva and pupa and longevity of sucrose fed female and male of *Aedes aegypti*; and the mean *Culex quinquefasciatus* male emergence time.

The percentage mortality of treated F1, F2 and F3 *Aedes aegypti* and *Culex quinquefasciatus* larvae after second exposure to LC_{50} decreased.

CHAPTER 1
INTRODUCTION

The innovation of wings has conferred great advantages to the predominance of small creatures such as insects over other land arthropods. Insects which attack man as well as other mammals are vectors of some of the most serious human diseases (Busvine, 1980). These diseases are grouped as viral, protozoa and nematode (Miyagi & Toma, 2000). Mosquito borne diseases include malaria, yellow fever, dengue, dengue hemorrhagic fever, Japanese encephalitis, filariasis.

Mosquitoes, the bloodsucking nuisance creatures have long been known for their importance as pests and vectors and found worldwide. They belong to the family Culicidae which is divided into 3 subfamilies: Toxorhynchitinae, Culicinae and Anophelinae. Among these, Culicinae and Anophelinae are the major groups of mosquitoes found living in tropical and sub-tropical regions of the world.

Anopheles, *Culex*, *Aedes*, *Mansonia*, *Haemagogus*, *Sabethes* and *Psorophora* are genera of mosquitoes that are medically important because of their blood sucking behaviour on humans (Abu Hassan & Yap, 1999). They can transmit several diseases such as malaria, filariasis, dengue, yellow fever and encephalitis. However, these diseases are more widespread in the tropics than in the temperate countries. In temperate climates, the life cycle of pathogens will be prolonged by the cold weather. Shorter life span mosquitoes usually often die before pathogens become infective (Busvine, 1980) compared to the tropical country.

Because of the direct annoyance from mosquito such as biting activities and transmission of diseases, various kind of household insecticides have been applied to kill or expel them. Some species of mosquitoes are known to be vectors of more than one disease or many diseases can be transmitted by more than one species of mosquito (Miyagi & Toma, 2000). The occurrence of transovarial dengue virus in wild population has been confirmed by Rohani et al. (1997) and this is the first report of transovarial transmission of dengue virus in Malaysia. Hence, the control of mosquito at the larval stage is important (Lee, 2002).

Mosquito control can be divided into four categories namely source reduction and environmental management, biological control, chemical control and physical barriers and personal protection (Yap & Zairi, 1999a). Chemical control is the main choice for vector control and will continue to be used in the near future because of its rapid action and limited development of alternatives. Most of the active ingredients in household insecticide products and repellent for mosquito control are made up of synthetic pyrethroids and only a few contain carbamate, organophosphate or organochlorine as active ingredients (Yap et al., 2000a).

The most effective methods to control the vector are adulticiding and larviciding. The chemicals used in adulticiding are mainly pyrethroids, which are highly effective against mosquito and show low toxicity to non-target organisms (Lee, 2000). Though sand granule of temephos was the larvicide used for more than two decades, low temephos resistance was reported (Lee et al., 1984; Lee & Lime, 1989) compared to malathion (Lee et al., 1987).

There are about 100 organophosphate insecticides reviewed to control disease vectors (WHO, 1986a). Organophosphate which contains carbon, hydrogen and phosphorus was reported to have low residual effect against insect and high mammalian toxicity (Metcalf, 1955). Malathion, temephos, fenthion, fenitrothion and chlorpyrifos are the organophosphate insecticides listed by WHO (1997b), which are suitable to use as larvicides to control mosquito due to its chemical unstable and non-persistent in environment (WHO, 1997c; Lee et al., 2003).

Abate, the organophosphate larvicide which contains temephos has been used in human drinking water to control mosquito larvae while malathion was used as an adulticide in Malaysia since 1973. It has been shown to be effective against all species of mosquito larvae (Technical Information Abate, 1969). Abate is a cheap market product and is easily available to the public. Hence, the study of the efficacy and sublethal effects on laboratory strain urban mosquitoes will provide efficient monitoring and the possibility detecting resistance development of the field strain mosquitoes.

This study was initiated with the following objectives:

1. To evaluate the efficacy of Abate against late third or early fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.
2. To determine the sublethal effects of Abate against *Ae. aegypti* and *Cx. quinquefasciatus* larvae *.*
3. To examine the mortality of F1, F2 and F3 *Ae. aegypti* and *Cx. quinquefasciatus* larvae after second exposure to sublethal concentration of Abate.

CHAPTER TWO
LITERATURE REVIEW

2.1 Taxonomy

The most acceptable taxonomic classification is by Knight and Stone (1977). According to them, the classification for *Aedes aegypti* (Linn, 1672) and *Culex quinquefasciatus* (Say, 1923) is as shown below:

Filum : Arthropoda

Class : Insecta

Order : Diptera

Famili : Culicidae

The family Culicidae is divided into:

1. Subfamily : Culicinae

   Tribus : Aedini

   Genus : *Aedes*

   Species : *Aedes* (*Stegomyia*) *aegypti*

2. Subfamily : Culicinae

   Tribus : -

   Genus : *Culex*

   Species : *Culex* (*Culex*) *quinquefasciatus*

2.2 The status of urban mosquitoes in Malaysia

Wide spread of mosquito is due to man's carelessness on the environmental conditions and hygiene. The rapid urbanization and transportation, population growth in the cities, greater use and disposal of non-biodegradable containers and poor living conditions with inadequate water supply in squatter areas has increased the incident rate of dengue cases in Malaysia since 1989 (Tham, 1997). Zainol (2000) reported that construction sites, cemeteries, building designs and vegetation around buildings provide breeding places for mosquitoes. Many cases of the dengue outbreaks were related and traced to the breeding of *Aedes* mosquitoes at construction sites (MOH, 1997).

In Malaysia including Sabah and Sarawak, there are 434 species representing 20 genera of mosquito fauna (Abu Hassan & Yap, 1999). *Ae. aegypti* and *Cx. quinquefasciatus* are two of the main urban mosquitoes that are found in Malaysia. *Ae. aegypti* is known as an indoor breeder while *Ae. albopictus* is an outdoor breeder. *Ae. aegypti* is the epidemic vector while *Ae. albopictus* is an occasionally epidemic vector of dengue viruses in parts of Asia (CDC, 2000).

Ae. aegypti is believed to have originated from the forests of tropical Africa (where the only true wild populations exist), to our ports via ships, and then spread along the coast by barter boats, and inland by other means of transportation. Initially, *Ae. aegypti* was found only in the coastal towns (Daniels, 1908; Leicester, 1908). It was reported to have moved inland and by

1920 was abundant in Kuala Lumpur. Lee & Hishamudin (1990) documented that it's spread in Peninsular Malaysia is complete. The reporting of clinical dengue cases have been on the increased since 1990 (Table 2.1).

Culex quinquefasciatus is the most common species found in Malaysia (Yap, 1992). The main factor supporting the existence of this species is probably due to poor sanitation cause by human migration to urban areas (Highton & Van someron, 1970). There is no surveillance method for this nuisance mosquito. They are the main cause of the mosquito bites in the evenings and night time that residents of housing estates complaint about (Mak, 2000).

These vector-borne disease cases can be reduced through improving the urban service and facilities such as domestic solid waste management, drainage system and sewerage system (Mohd Ridhuan, 2000).

2.3 Medical importance of mosquitoes

Among the 20 mosquito genera found in Malaysia, *Aedes*, *Culex*, *Anopheles* and *Mansonia* are the four main groups that contain species of medical importance. Among them, the mosquitoes transmit diseases such as dengue fever/dengue haemorrhagic fever (DHF), malaria, urban filariasis, rural filariasis and Japanese B-encephelitis. Since the 1950s, dengue fever (DF) and dengue haemorrhagic fever (DHF) have been the most common urban disease in Southeast Asia and are now considered as worldwide diseases (Yap et al., 1994a).

The total number of dengue fever cases in Malaysia increased from 10146 cases in 1999 to 32422 cases in 2004 (Table 2.1). The total number of deaths also increased to 102 cases in 2004 from 37 in 1999. It has become a nationwide concern in Malaysia.

Table 2.1: Number of total dengue fever cases and deaths in Malaysia

Year	No. of total dengue fever	No. of deaths
1999	10146	37
2000	6692	45
2001	15446	50
2002	30807	99
2003	30221	72
2004	32422	102
2005	16861	107

- Reported by WHO, DengueNet (2006).

Ae. aegypti is an important vector in Southeast Asia and is a domestic mosquito which prefers to breed indoors in artificial containers in rural and urban areas. It transmits diseases such as yellow fever, dengue and dengue hemorrhagic fevers (DF/DHF). Currently, there is no effective vaccine against dengue and dengue hemorrhagic fevers and therefore the most important method of control is against the vectors.

Cx. quinquefasciatus is the most abundant and ubiquitous mosquito and is also known as the tropical house mosquito (Abu Hassan & Che Salmah, 1990). According to Harwood & James, (1979), Sucharit (1988) and Lane & Crosskey (1993), *Cx. quinquefasciatus* is one of the major vectors for human lymphatic filariasis (urban filariasis) caused by the nematode *Wuchereria bancrofti*.

2.4 Insecticide resistance in mosquitoes

The word "resistance" is used in instances where insecticide dosages that were formerly effective now meet with control failure. It was initially difficult to realise that this event was due to a change in the susceptibility of the insect population, and indeed when the phenomenon first appeared it was then and still is necessary to test not only the quality of the insecticide then used but also thoroughness of its application. The problem of resistance can only be identified when the quantitative methods had been worked out for testing the insects themselves (Brown, 1958).

Resistance is defined as the acquired ability of an insect population to tolerate doses of insecticide which will kill the majority of individuals in a normal population of the same species (WHO, 1975). Resistant status can be evaluated according to the WHO protocol. A population is considered resistant if more than 20% survives the diagnostic dose compared to the susceptible colony (WHO, 1981).

Pesticide resistance is an increasingly worldwide problem. Through biochemical research, it has been found that resistance depends on one or more mechanisms which protect the insect from the effects of the insecticide (Busvine, 1980). The wide use of synthetic insecticides has caused the emergence of resistant strains through the widespread and persistent destruction of susceptible mosquitoes. The tolerant mosquito may survive. The resistant strain is a selection of pre-existing genetic types, analogous to the selection of new types and varieties by natural selection (Busvine, 1980).

According to Georghiou (1990), a 5 year study conducted in El Salvador showed that continued severe selection pressure would eventually result in the evolution of such high resistance that the mosquito population could no longer be suppressed effectively by agricultural sprays. The data showed that the adult mosquito population within the cotton area was suppressed only mildly by the first treatments and that its density recovered steadily, so that by the end of the spraying season it had reached a level similar to that observed in the non-cotton area. It has been observed that the problem of vector resistance is more acute in agricultural areas that are heavily treated with insecticides. In other words, mosquito populations are more resistant in agricultural than in nonagricultural areas even when both areas have received an equal number of treatments by public health authorities (Georghiou, 1990).

According to Busvine (1980), among culicinae mosquitoes, the members of the widely dispersed *Cx. pipiens* group have developed resistance to various groups of insecticides. Ferrari & Georghiou (1990) also reported that selection by toxic substances can increase the amount of enzymes that are responsible for detoxification. Recently, the findings of a study suggested that a single genetic mutation was responsible for the development of resistance to a major class of insecticides in disease-carrying mosquitoes. Mosquitoes will rapidly develop resistance to organophosphate and carbamates, especially in urban areas where they are frequently applied (Carlsson, 2003). These insecticides function by blocking a key enzyme, acetycholinesterase, in the insects' nervous system which paralysed and consequently result in death.

Chemical control has accelerated the development of mosquito resistance and brought a lot of the toxic hazards to human, livestock and other wildlife (Busvine, 1980). The impact of resistance is an indicator of the potential hazards of our present practice of using insecticide extensively. Despite the potential for developing new insecticides, chemical warfare is unlikely to overcome the insects; drugs and vaccines should continuously be developed to solve the infectious disease problem carried by mosquitoes (Weill et al., 2003).

2.5 Biology of mosquitoes

2.5.1 *Aedes aegypti*

Ae. aegypti (Plate 2.1), the yellow fever and dengue mosquito, having black and white markings on the thorax and leg, can be commonly found in Asia, the Pacific region and parts of America. It transmits dengue, including the haemorrhagic form, and is a potential vector of urban yellow fever (Busvine, 1980). It is a dominant indoor breeder. This species can be recognized from the others by the black and white scale pattern on the dorsal surface of adult's thorax.

The eggs are black in colour and laid singly. They can always be found at wet places at the edge of the water surface in tree holes, mud, leaves on pond edges, rock pools and wet earthen jars (Abu Hassan & Yap, 1999). The eggs may withstand almost two years in dry habitats (Fox, 1974). It will hatch when submerged in water. The Institute of Medical Research (IMR), Kuala Lumpur reported that *Ae. aegypti* is able to lay about 102 eggs per female and takes 1 to 48 hours to hatch. The life span of larvae is 6 to 8 days and for pupa is 1 to 2 days. The life cycle takes about 9 to 10 days to complete. Longevity of female adults is higher, 12 to 56 days compared to male adults, 10 to 29 days (Vythilingam et al., 1992; Lee, 2000).

Plate 2.1: *Aedes aegypti*, the important vector of the yellow fever and dengue.

The *Ae. aegypti* larvae has short siphon and blunt with a pair of subventral tuft at the posterior and at least three pairs of ventral brush on the 9th segment. The larvae can be recognized by the combed teeth arranged in 2 or more rows (Abu Hassan & Yap, 1999). They are always found in clear water. It needs 5-12 days to transform into pupae (Sulaiman, 1990). The life cycle from the egg to adult stage usually takes 6-8 days. The biting activity peaks at dawn and dusk (Abu Hassan & Yap, 1999).

Female mosquitoes normally acquire pathogens during blood feeding, followed by incubation of the parasites for several days before they can be transmitted successfully to naive host (Detinova, 1986). Xue et al. (1995) suggested that older, infective mosquitoes contact more hosts than younger, noninfective mosquitoes. Their results also showed that older female *Ae. aegypti* generally require a larger blood meal compared to younger ones before subsequent feeding activity is inhibited and egg development is initiated.

Nasci (1986) found that larger *Ae. aegypti* either live longer or are more successful in locating hosts. He suggested that large females may play a more important role in the maintenance and amplification of mosquito-borne pathogens than small individuals.

Lee (2000) suggested that the widespread use of temephos in indoor containers had caused reduction in *Ae. albopictus.* This could be due to urbanization has encouraged *Ae. aegypti* to breed in outdoor containers whenever available. This changing pattern in the breeding habits of *Ae. aegypti* may be significant epidemiologically since it is a highly domestic mosquito, dependent on humans for blood.

2.5.2 *Culex quinquefasciatus*

Cx. quinquefasciatus (Plate 2.2) can be found in the tropics and subtropics and is also found distributed in temperate regions of North and South Hemisphere (Sucharit et al., 1989). It is the most common species found in Malaysia (Yap. 1992).

This urban mosquito is closely associated with humans, breeding near dwellings and feeds on them because they need blood meal to reproduce. Its larvae can be found in blocked and polluted stagnant water containing a high degree of organic matter such as sewerage waters (Pantuwatana et al., 1989, Yang et al., 1997, Abu Hassan & Yap, 1999) as a source of food (Ramalingam et al., 1968), close to human habitation.

This mosquito lays its eggs in rafts on water surface, in rain barrels, tanks, cisterns, catch basins, and other small collection of water (Herms & James, 1996). The eggs are usually brown, long and cylindrical, laid upright on the water surface and placed together to form an egg raft (Plate 3.6) which can comprise up to 300 eggs (Service, 1996). The eggs take 27 hours to emerge to first instar larvae (Sulaiman, 1990).

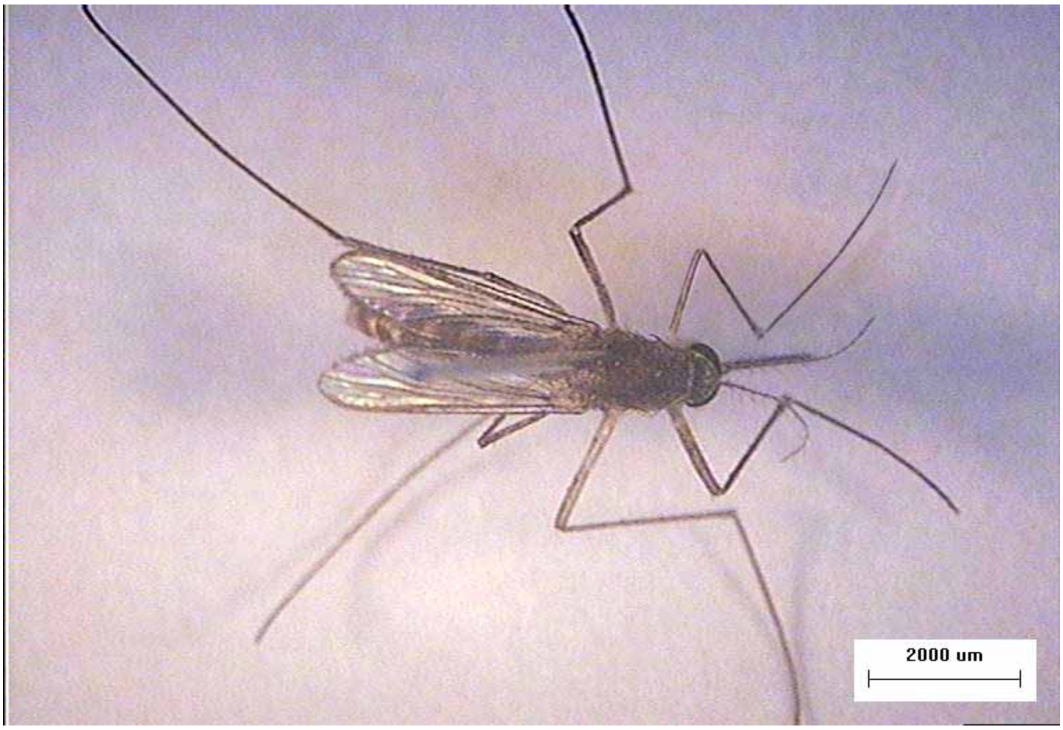

Plate 2.2: *Culex quinquefasciatus*, one of the urban pests in Malaysia.

The larvae have a long and narrow siphon on the eighth abdominal segment and several ventral brushes on the siphon and feed on microorganism below the water surface (Abu Hassan & Yap, 1999). It requires about 10 to 14 days to fully developing under warm summer conditions; egg stage 24 to 36 hours, larva about 7 to 10 days and the pupa about 2 days (Horsfall, 1955). Increase in daily temperature will also increase egg production (Hayes & Downs, 1980). This was confirmed by Reisen et al. (1990), who showed that the gonotrophic age in Burma was 2.75 days while in California it was 4 days.

In Pakistan, where the temperature is higher, the mosquito will just lay eggs at 5 days old (Suleman & Shirin, 1981). In contrast, in Florida, United States, the mosquito lay eggs at 6-9 days old (Weidhaas et al., 1971). Its life history is greatly influenced by temperature.

The larvae go through four developmental stages within a period of 7-8 days. They feed on microorganisms below the water surface. Any insecticide that floats on the water will be ineffective against larvae. Most of the adults are always dull in colour. Females readily enter houses at night and bite man in preference to other mammals (Sirivanakarn, 1976; Yap et al., 2000). After the blood meal, they will usually rest indoors or outdoors (Abu Hassan & Yap, 1999). The life cycle will continue after they have laid their eggs.

2.6 Chemical control of mosquitoes

Among the various pest control approaches, chemical control involving the use of insecticides appear to be the norm for both public health and household insect pest control (Yap, 1984; Yap, 1996; Yap, 1998; Yap & Foo, 1984; Yap et al., 1994, Yap et al., 2003). Chemicals used as insecticides for mosquito control include substances which destroy mosquitoes and are commonly known as pesticides or insecticides (WHO, 1996a).

Chemical control has become the most popular approach in mosquito control since the introduction of organic insecticides in 1940s (Yap et al., 2003). Organochlorines, organophospate, carbamates and pyrethroids are the popular insecticides used (WHO, 1997a). Conventional approaches such as adulticides, larvicides and integrated control are the usual control measures conducted.

Due to the cost involved, need for continuous input, and safety and environmental factors; chemical control by insecticides alone will seldom be the control method of choice for communities (Yang, 1982). Furthermore, insecticidal control will require specialized training in application, storage and handling of insecticides (WHO, 1996a).

2.6.1 Larvicide

A larvicide is an insecticide that is specifically targeted for the control of mosquito immature stages especially the larva (WHO, 1996b). Larviciding was recommended as a complementary measure to basic sanitation in 1984 (WHO, 1984). A suitable larvicide should have rapid and persistent action in different kinds of aquatic habitat where mosquitoes breed such as polluted and brackish water as well as fresh water (WHO, 1996a). The WHO Expert Committee on Malaria (WHO, 1957) considers that the use of larvicides should be restricted to situations where residual spraying is ineffective owing to the particular habits of the vector or uneconomical because of the great number of houses involved.

Latest used of various contact poisons have replaced the oiling formulation of earlier control measure of mosquito larvae. Larvicide formulation can be divided into granular preparations, emulsifiable, concentrates and wettable powders and solutions (WHO, 1972). Larvicide such as temephos, fenthion, chlorpyrifos, alpha-cypermethrin, etofenprox, fenitrothion, permethrin and cyfluthrin have been used. Larvicide is more easier to handle compare to adulticide, less expensive and no or limited residual action (WHO, 1996a).

2.6.1.1 Characteristics of Temephos

Mosquito larvae breeding in water thickly set with reeds and other aquatic vegetation present difficulties of penetration to liquid or dust larvicides. A convenient and effective treatment for such sites is to scatter insecticidal pellets (0.6-2 mm) or granules (0.25-0.6 mm) (Busvine, 1980).

The major larvicide used in the control of the dengue vector *Aedes aegypti* Linnaeus is the organophosphate temephos (Bang & Pant, 1972; Teixeira et al., 1999). It is added to domestic stored water for the control of *Aedes* breedings since it is neither accumulated in the treated water nor harmful to man (Laws et al., 1967)

The control of *Ae. aegypti* in Malaysia depends primarily on the use of malathion as adulticide, and temephos (Abate®) as larvicide (Lee & Lime, 1989). According to Cheong in 1978, since the major DF and DHF outbreak in 1973, the regular application of temephos (Abate®) in Malaysia is one of the major methods of controlling both *Ae. aegypti* and *Ae. albopictus*.

Temephos (Abate®) or 0,0,0',0'-tetramethyl, 0,0'-thiodi-p-phenylene phosphorothioate is an organophosphate compound characterized by very low toxicity to warm blooded animals (Lee & Lime, 1989). The empirical formula is $C_{16}H_{20}O_6P_2S_3$ (Figure 2.1). The molecular weight is 466.5 g. It was introduced in 1965 by American Cyanamid Co. under the code name AC 52160 and its trade marks are "Abate", "Abathion", "Abat", "Swebat", "Nimitex" and "Biothion". Pure temephos is a white crystalline solid, melting point 30.0 – 30.5°C while the technical grade is a brown visquous liquid 85-90% pure. It is almost insoluble in water but soluble in acetonitrile, carbon tetrachloride, diethyl ether, 1,2-dichloroethane, toluene and lower alkyl ketones. It is stable in 25°C and the optimum stability is at pH5-7 (WHO, 1988).

The WHO Expert Committee on Insecticides authorized the use of temephos in drinking water at a target dose of 1 mg/l (WHO, 1973). Temephos is an organophosphorus (OP) compound which has low mammalian toxicity and not harmful to human when used at operational dosages. The oral toxicity of rats, LD_{50} is 8600 mg/kg (Chavasse & Yap, 1997). Temephos has been used in human drinking water to control *Ae. aegypti* because of its low mammalian toxicity. It has also used to control human body lice and fleas on cats and dogs.

Vector control services (outside of North America) dealing with *Ae. aegypti* frequently employ temephos (Abate) sand granules for treatment of containers holding potable water (Curtis & Minjas, 1985). The chemical insecticide functions by entering the insect's body by ingestion through the mouth (WHO, 1996a). Hence, it works by inhibiting the activity of cholinesterase enzymes at the neuromuscular junction, which caused paralysis and death (Florida Coordinating Council on Mosquito Control, 1998).

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2.6.2 Adulticides

Adulticides are specifically used to control adult mosquitoes whether they are flying or resting (Yap et al., 2000a). The chemicals are dispensed out by space spray and surface residual spray. However, epidemics, emergencies and public demand may require adulticiding especially space spraying (WHO, 1996a).

Space spraying is defined as the destruction of flying mosquitoes by contact with insecticides in the air (WHO, 1996a). The objective of space spraying is to prevent adult female mosquitoes; in particular, disease infected mosquitoes from biting human beings. The scope of space spraying includes indoor and outdoor application of insecticides. In Malaysia, thermal fogging and ULV are normally conducted only in epidemic areas (Yap et al., 2000). Not long lasting effect, cost, contamination and pollution are the disadvantages of space spraying (WHO, 1997b).

In Malaysia, the insecticide malathion is used routinely as adulticide while Abate (temephos) is one of the important chemical agents used widely as larvicide since 1973. It is cheap and easily available and used as a sand granule formulation containing 1 % active ingredient and is added to potable water at a dosage of 10 g per 20 gallon or 90 liter of water. This would make the final total release dosage as 1.11 mg active ingredient per liter or 1.11 ppm (Lee, 1990). The residual efficacy of Abate in any formulation depends on the aquatic habitat and the water quality. According to the technical information reported by American Cyanamid Company in 1969, 1 ppm of Abate was found to control the mosquito larvae effectively for 3 to 4 months in potable water.

2.6.3 Insect growth regulator

Substances which inhibit the normal growth of mosquito larvae resulting in early death are insect growth regulators. They are synthetic organic chemicals, categorised under biological control and are used in the same way as other larvicides. However, they are not directly toxic to mosquitoes. Mortality is caused by the inability of the mosquito to complete metamorphosis (WHO, 1996a). For *Ae. aegypti* and other urban mosquitoes, larviciding is more often used than adulticiding (WHO, 1996a).

2.6.4 Microbial agent

Microbial agents such as *Bacillus sphaericus* and *Bacillus thuringiensis* H14 are also used to control the larvae. *Bacillus sphaericus* Neide, a spore-forming bacterium has received much attention for possible development as a mosquito larvicide (Mulla, 1986; Mulla & Singh, 1991). It has shown excellent biological activity against several genera of mosquitoes, but it is more specific and highly active against *Culex* mosquitoes (Davidson et al., 1984, Lacey & Singer, 1982, Lacey et al., 1984, Mulla et al., 1984, Mulla et al., 1986).

2.6.5 Integrated control

Integrated control using both chemical and biological agents against both adult and larval mosquitoes is the latest approach for the overall mosquito control (Yap, 1998; Yap et al., 1994b). *Bacillus thuringiensis* H-14 (VectoBac AS 12) and two adulticide formulations (Aqua-Resigen or Pesguard PS 102) in a ULV formulation has been used as a recent integrated control of common vector mosquitoes (Tidwell et al., 1994; Yap et al., 1997b, Yap et al., 1997c).

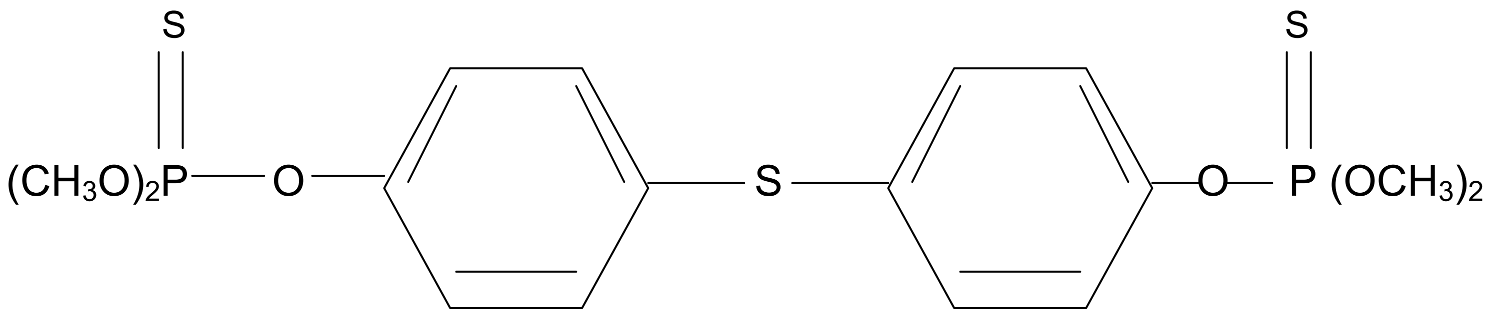

Figure 2.1 Structural formula of Abate